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Prevalance and Antibiotic Susceptibility Testing Of Staphylococcus Saprophyticus in Urine Specimens Among Reproductive Age Group Females.

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Abstract:
Background*- Staphylococcus saprophyticus is a gram-positive coagulase negative staphylococcus[CONS]. It is the second most frequent cause of urinary tract infection[UTI] in reproductive age group females(15-40years) after Escherichia coli (70.8%). The bacterium has capacity for selective adherence to human urothelium. S.saprophyticus is innately resistant to novobiocin which is used as a screening tool to detect it in urine specimens.*
Material and method*- The study was conducted over a period of 6months from August 2018-January 2019. 80 mid-stream urine samples were collected from reproductive age group females attending obstetrics and gynaecology OPD with or without symptoms of UTI. CONS were identified by gram staining of culture smears and standard biochemical tests. Novobiocin susceptibility test was done using Kirby-Bauer disc diffusion method as per CLSI guidelines to identify S.saprophyticus species.*
Result-Out of 80 urine samples which were processed,12[15%] were positive for S.saprophyticus. Highest resistance was seen for ampicillin and maximum sensitivity was seen for vancomycin.
Conclusion*-CONS are normal inhabitants of human skin and mucous membranes. They have long been dismissed as culture contaminants, but now the potentially important role of CONS as pathogens and their increasing incidence has been recognised particularly S. saprophyticus should be considered among agents causing UTI in women 15 to 44 years old. Novobiocin susceptibility test,a screening tool to detect S.saprophyticus in urine specimen is a rapid,simple and reliable method.*
Keyword: *Staphylococcus saprophyticus,novobiocin resistance,urinary tract infection,Coagulase Negative Staphylococcus ,antibiotic susceptibility test.*

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I. Introduction

Staphylococcus saprophyticus is a gram-positive,coagulase negative staphylococcus[CONS] belonging to family Micrococcaceae.It is the second most common pathogen after Escherichia coli causing 10-20% of allurinary tract infections [UTI] in sexually active young women [1,2]. Gastrointestinal tract is the major reservoir of S. saprophyticus. Rectal, vaginal, and urethral colonization of S. saprophyticus was associated with UTI caused by this organism[3]. The virulence factors include adherence to urothelial cells by means of a surface-associated protein, lipoteichoic acid; a hemagglutinin that binds to fibronectin, a hemolysin; production of extracellular slime[4]. It possess enzyme urease that hydrolyze the urea to produce ammonia, main virulence factor for UTIs. Apart from urease activity it has numerous transporter systems to adjust against change in pH, osmolarity, and concentration of urea in human urine. Young women are more susceptible to genitourinary colonization in association with hormonal influences that occur near or during menstruation. UTI caused by S. saprophyticus is associated with recent sexual intercourse and occurs more often during late summer and fall [3]. Alterations in the genital flora effected by spermicides or candidal infection favor colonization by S. saprophyticus[6]. S.saprophyticus is intrinsically resistant to novobiocin. Testing of novobiocin susceptibility is reported to be 100% sensitive and 96% specific [7]. This provides a simple and reliable screening method to differentiate the presence of *S. saprophyticus* from the presence of other CONS.

S. saprophyticus isolates has increased antimicrobial resistance when these microorganisms were grown in biofilms, suggests that it is a very important virulence factor for S. saprophyticus, which permits this species to establish persistent UTIs[8]. S.saprophyticus is resistant to the drugs most often used for the empirical treatment of UTI[9]. 17.6% of the S. saprophyticus isolated from UTIs tested by Ferreira et al. were resistant to sulfamethoxazole/trimethoprim, a fact that may lead to therapeutic failure when UTIs are treated empirically. Antimicrobial susceptibility testing of these strains is therefore necessary[10]. The current study aims to know

the prevalence and antibiotic susceptibility pattern of Staphylococcus saprophyticus in urine samples among reproductive age group females[14-40years].

II. Material and Methods

Mid-stream urine samples from 80 subjects were collected over a period of 6months. Reproductive age group [14-40years] females attending Obstetrics and gynaecology OPD with or without urinary complaints were included in the study.

Inclusion criteria-

1. Young females belonging to reproductive age group (15-45 years) attending Obstetrics and gynaecology OPD with or without urinary complaints.
2. Individuals with no prior antibiotic usage in past 3 weeks.

Exclusion criteria-

1. Patients on urinary catheter.
2. Patients who are admitted in hospital.
3. Individuals who had antibiotic usage within past 3 weeks.

Methodology- A clean-catch midstream urine[MSU] sample was collected in a sterile wide mouth container labeled with information on the patients age, sex, and brief clinical history. The samples were transported immediately to the laboratory, Upgraded department of Microbiology, OGH,Afzalgunj and processed for culture and antimicrobial drug susceptibility as per the routine microbiological techniques. Semi-quantitative urine culture using a calibrated loop[(0.001 ml MSU] was done to isolate the pathogen on blood and MacConkey agar as per the recommendations of Kass.[11] The plates were incubated at 37°C for 24 h and further incubated for 48 h in culture (growth) negative cases. Following this, the isolates were identified by standard biochemical tests, and diagnosis of UTI was made when pathogens were present at a concentration of at least 10^5 colony-forming unit (CFU)/ml of urine. Gram staining of culture smears was done for the assessment of purity and observation of their specific morphology. After confirmation of presence of GPCs, the strains were submitted to the catalase and tube coagulase (gold standard) tests to distinguish Staphylococcus aureus and coagulase-negative staphylococci (CoNS) as recommended by Koneman et al. (1997).

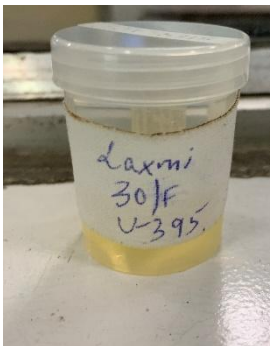


Fig1 depicts container for collecting MSU with patient detail's label.

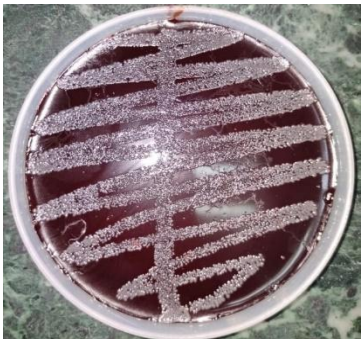


Fig2 depicts semi-quantitative culture of MSU on blood agar showing 10^5 CFU/ml

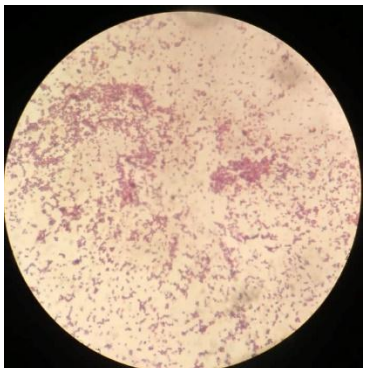
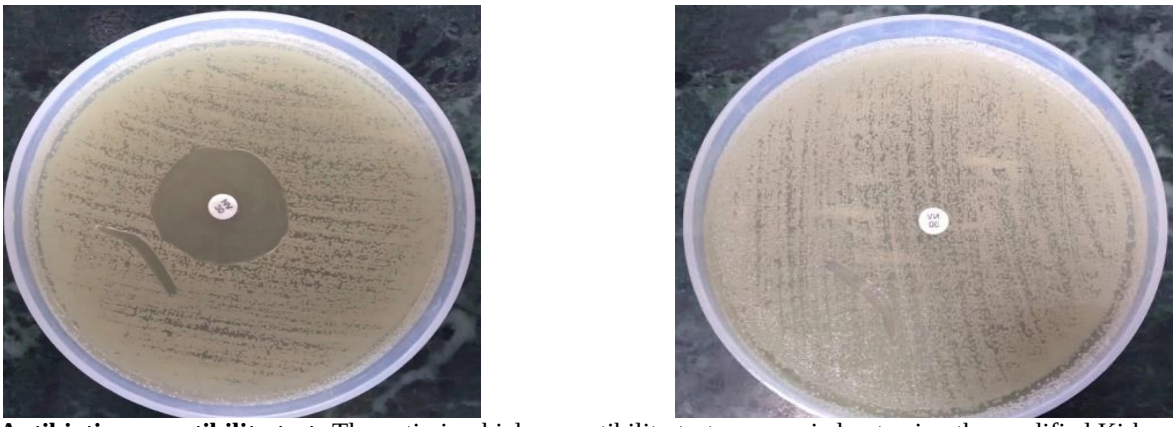


Fig 3 shows GPC in clusters.

Fig 4 depicts tube coagulase test. Left- tube coagulase negative. Right- tube coagulase positive

Isolation of Staphylococcus saprophyticus -Coagulase negative isolates were processed further by novobiocin susceptibility test as per CLSI guidelines. Subcultures equivalent to 0.5 McFarland opacity standard was inoculated by lawn culture method on Mueller Hinton agar and 5ug novobiocin disc was placed. Zone of Inhibtion-16mm or more were considered sensitive and less than 11mm or no inhibition were considered resistant.



Antibiotic susceptibility test- The antimicrobial susceptibility test was carried out using the modified Kirby-

Fig 5 Depicts Novobiocin susceptibility test. Left- sensitive to Novobiocin(ZOI>16mm). Right- resistant to Novobiocin(ZOI <11mm) suggestive of S.saprophyticus

Bauer disc diffusion technique[12]. The standard suspension of each isolate that matched 0.5 McFarland standard was used to swab over dried Mueller Hinton agar plate and the following discs were placed on the plates after 20 min of inoculation: Ampicillin 10 µg, Amoxyclav 30 µg, Tetracycline 30 µg, Cefepime 30 µg, Cefoxitin 30 µg, Nitrofurantoin 300 µg, Gentamicin 10 µg, Ciprofloxacin 5 µg, Co-trimoxazole 25 µg, Linezolid 30 µg and Vancomycin 30 µg.The isolates were screened for Methicillin resistant staphylococcus saprophyticus[MRSS] using 20 µg Cefoxitin. The plates containing the discs were allowed to stand for at least 30 min before incubation at 35°C for 24h. The diameter of the zone of inhibition produced by each antibiotic disc was measured and interpreted using the CLSI zone diameter interpretative standard.

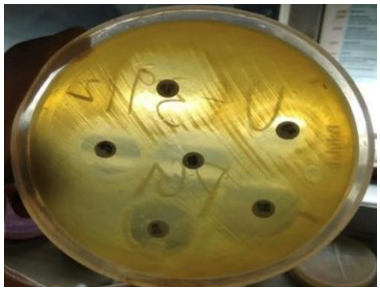
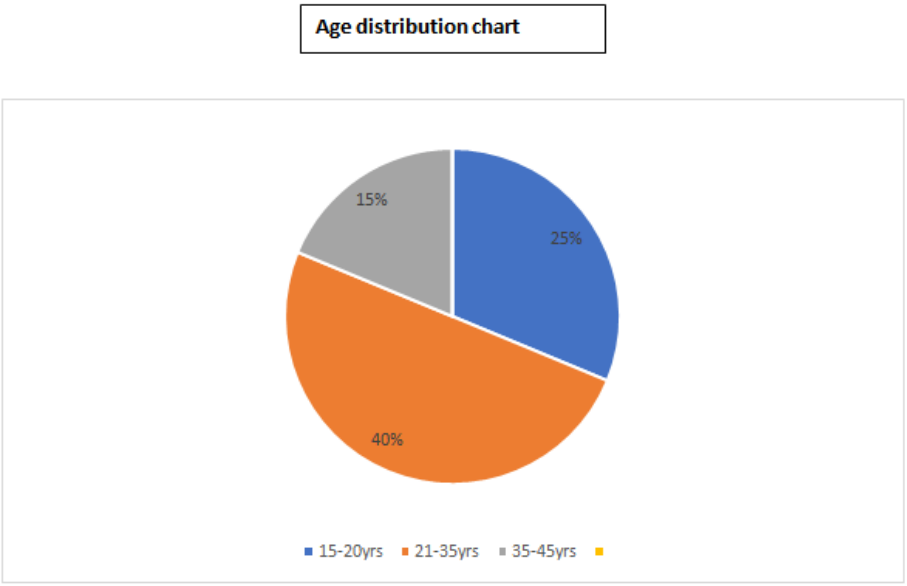


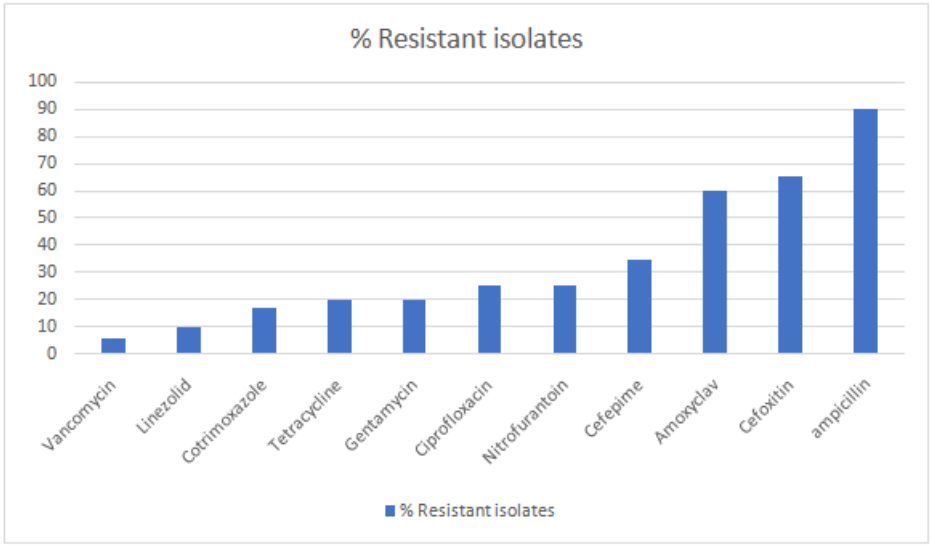
Fig 6 shows AST plate with six drugs and their zones of inhibition.

III. Results

Out of the 80 samples, S. saprophyticus were isolated from 12[15%] samples. 25 females were pregnant and had symptoms of UTI. 10 had complaints of dysuria or frequent micturition and the rest 45 attended OP for complaints of menorrhagia,irregular menstrual cycles or infertility without any urinary complaints. Maximium resistance was seen to ampicillin and cefoxitin[used for screening of methicillin resistant staphyloccus saprophyticus,MSSS]. Majority of the isolates were sensitive to floroquinolones,trimethoprim-sulphmethoxazole,vancomycin.

No of patients	Associated condition
10	Complaints suggestive of UTI such as dysuria,frequency,urgency,suprapubic tenderness.
25	Pregnant women. 15 Pregnant women with complaints suggestive of UTI 10 Pregnant women without complaints of UTI
45	Complaints of oligomenorrhea,menorrhagia,infertility





IV. Discussion

CONS were considered to be urinary contaminants prior to the 1960s. In 1962, Torres Pereira reported the isolation of coagulase-negative staphylococci possessing antigen 51 from the urine of women with acute UTI[13]. In subsequent years, additional reports supported this concept [14]. The organism was found to belong to micrococcus subgroup 3. It was later reclassified as S. saprophyticus. Many UTIs were treated with an empirical antibiotic therapy that was ineffective for S. saprophyticus, revealing that S. saprophyticus is an aetiology that is insufficiently considered in UTI. Wallmark et al. isolated S. saprophyticus from the urine of 173 of 787 (22%) consecutive female patients found to have bacteriuria. The highest rate of S. saprophyticus infection was 42.3%, among women aged 16–25 years included in the study[15]. Gupta et al. reported a prevalence of 8%, Prakash and Saxena reported a prevalence of 9.68%, Foxman B et al. reported 42%

S.saprophyticus in UTIs in young females[16,17,18]. The prevalence of S. saprophyticus was 15% in our study similar to a study conducted in Australia by Schneider et al, where it was 15.2%[19]. Maximium number of our isolates were sensitive to floroquinolones,trimethoprim-sulphmethoxazole,vancomycin and resistant to beta-lactams. In a similar study Khoshbakht et al. shown that S. saprophyticus isolates, as the most frequent Gram positive bacteria in UTIs, exhibited high resistance to ampicillin, tetracycline and erythromycin (92.31%) and high susceptibility to nitrofurantoin and vancomycin (92.3%)[20].Martinez et al reported almost half of S. saprophyticus strains were considered oxacillin-resistant, thereby denying the benefit of treatment with oral beta-lactams in urinary tract infections.[21] The administration of fluoroquinolones is recommended for uncomplicated UTIs in areas where the incidence of trimethoprim/sulfamethoxazole resistance is higher than 10%.[22]. Fluoroquinolones have been successfully used to treat a wide range of community-acquired and hospital-acquired infections, and rates of resistance to fluoroquinolones remain low.[23]

V. Conclusion

CONS are commensals of human skin and mucous membranes. S.saprophyticus should be considered among agents causing UTI in women aged 15 to 44 years. Young sexually active women are more susceptible to genitourinary colonization by Staphylococcus saprophyticus. Novobiocin susceptibility is a simple and inexpensive test which is 100% sensitive and 96% specific. More than half of S.saprophyticus strains are methicillin-resistant, thereby denying the benefit of treatment with oral beta-lactams in urinary tract infections. Routine antimicrobial susceptibility testing of these strains is necessary to avoid therapeutic failure.

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